

# TWO NEW SPECIES OF MICROSPORIDIAN PARASITES FROM A MARINE FISH *SAURIDA TUMBIL*

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## ABSTRACT

Two new species of microsporidian parasites, *Pleistophora sauridae* n.sp. and *Nosema sauridae* n.sp. parasitising visceral muscles of the marine fish, *Saurida tumbil* have been described. The infection is light and occurs throughout the year. The parasites are apparently harmless to the host. These are the first records of microsporidian parasites from the present host.

## INTRODUCTION

DURING the period 1968-70 our attention was drawn to the occurrence of small cysts attached to the visceral muscles of a local marine fish, *Saurida tumbil*. The cysts on examination proved to be the developmental stages of two microsporidian parasites which for reasons discussed elsewhere in this paper are considered new to science, and the names *Pleistophora*\* *sauridae* n.sp. and *Nosema sauridae* n.sp. have been proposed for them.

## MATERIAL AND METHODS

The host fish, *Saurida tumbil* were collected from fish landings of the offshore fishing station at Visakhapatnam (Andhra). When the fish was opened whitish, opaque cysts measuring 1-2 mm in diameter, were seen attached to the visceral muscles and could be dislodged easily. Smears of cysts showing spores were examined in the fresh condition and measurements taken. The polar filaments were released by the addition of a drop of hydrogen peroxide to the wet smear under pressure of a cover slip and

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\* Sprague and Vernicke (1968) state that according to the law of priority (Art. 23) we must regard *Pleistophora* Gurley as the valid name and discard its junior synonym *Plistophora* Labbe.

were examined under dark ground illumination. Smears were fixed in methyl alcohol and stained in Giemsa. Material for sectioning was fixed in Alcoholic Bouin's fluid, sectioned at  $8\mu$  thickness and stained with Heidenhain's iron haematoxylin. The spores were also stained according to Feulgen's technique and Periodic acid-Schiff technique.

#### OBSERVATIONS

*Pleistophora sauridae* n.sp.

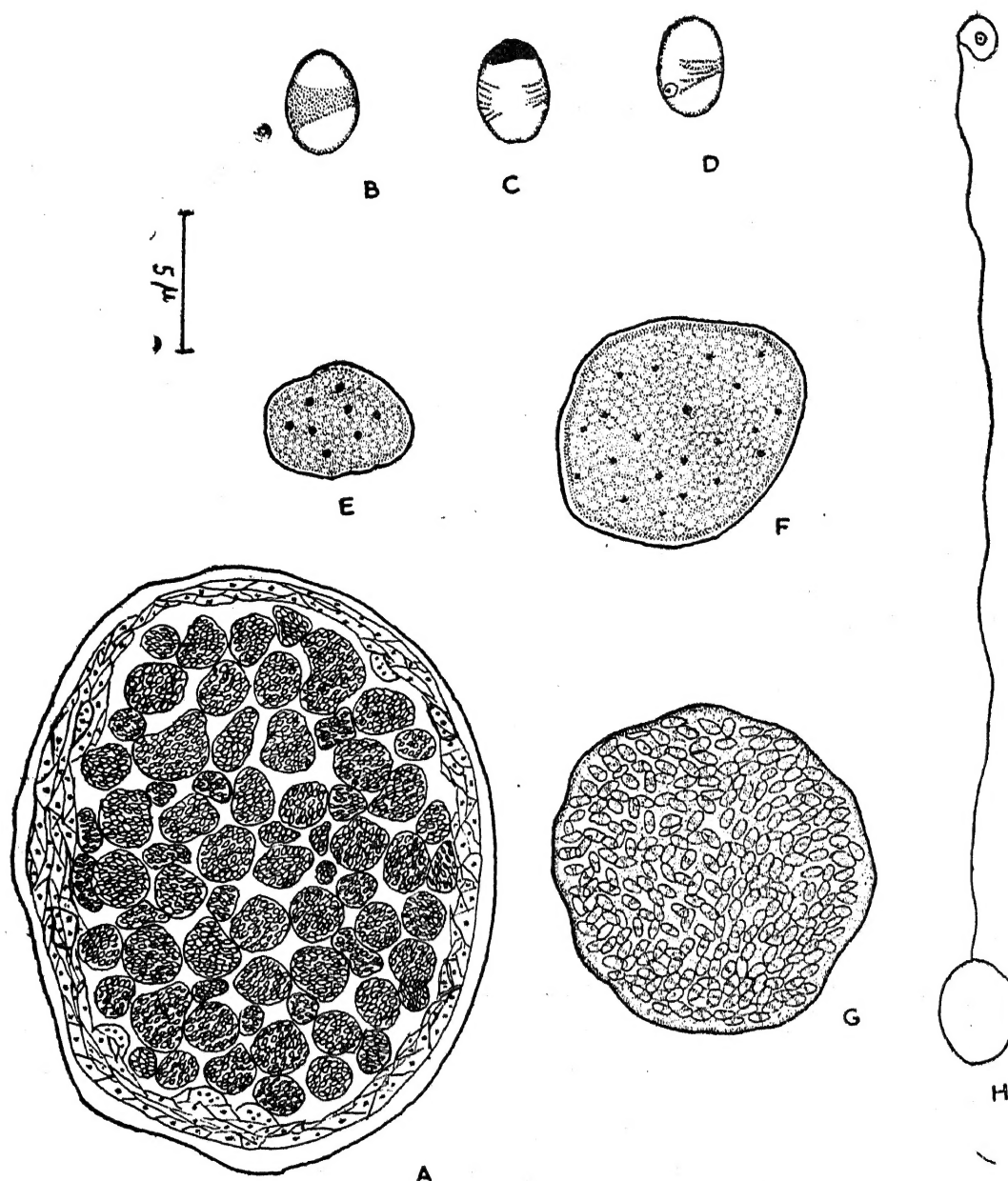
Host: *Saurida tumbil*

Site of infection: Visceral muscles.

Type slide: Department of Zoology, Andhra University, Waltair.

Infection by the parasite was found throughout the year. The infection was always light, each host not yielding more than ten cysts. The cysts are surrounded by a layer of host tissue. No apparent inflammation, hyperplasia or injury to the host tissue has been observed. The sporonts in the cysts were in different stages of development. The earlier stages were found towards the periphery while the more advanced stages were in the centre of the cyst. Sporonts having varying number of nuclei and having an irregular outline have been observed in the periphery of the cyst; thus a sporont measuring  $6.0\mu \times 4.0\mu$  showed eight nuclei and others measuring  $10.0\mu \times 8.0\mu$  showed twenty-one nuclei (Figs. 1 E & F). In the mature cysts, the pansporoblasts each containing several spores were found in the centre of the cysts (Fig. 1 A). The wall of the pansporoblast is very thin and owing to the pressure of the other developing pansporoblasts the wall may sometimes rupture releasing the spores into the cyst giving it the appearance of a *Nosema* cyst.

The spores are oval and refractile in fresh smears (Fig. 1 B). The measurements of one hundred spores taken at random have a range of length from  $3.6\mu$ – $4.2\mu$  and a breadth of  $2.0\mu$ – $2.2\mu$ . Two vacuoles, an anterior smaller and a posterior larger one with the cytoplasm extending in the form of a pale band in between the vacuoles can be seen in fresh smears (Fig. 1 B). The polar filament is uniformly thin and when fully extruded measures  $50.0\mu$  in length (Fig. 1 H). When the spores are stained according to the PAS technique a small PAS positive cap is seen lying in front of the anterior vacuole (Fig. 1 C). Spores stained with Giemsa after an initial hydrolysis in 1 N HCl for 10 minutes revealed the polar filament with 6–8 coils within the spore (Fig. 1 D). Feulgen stained spores showed a single dot-like nucleus in the sporoplasm.



**FIG. 1. PLEISTOPHORA SAURIDAE N.SP.**

FIG. 1. *Pleistophora sauridae* n. sp. (A) Section of a cyst showing the outer muscular and an inner epithelial layer enclosing the pansporoblasts. (B) A fresh spore: Note the sporoplasm in the form of a band in the middle. (C) A spore stained according to the PAS technique: Note the helmet shaped polar cap at the anterior end. (D) A spore stained by Giemsa: Note the coils of the polar filament seen within the spore. (E) A sporont showing eight nuclei. (F) A sporont showing twenty-one nuclei. (G) A single pansporoblast showing numerous spores. (H) A single spore showing the extruded polar filament.

## DIAGNOSIS

The present form parasitising the visceral muscles of *Saurida tumbil* belongs to the genus *Pleistophora* because each pansporoblast produces more than sixteen spores. So far sixteen species of *Pleistophora* have been reported from fishes. Putz *et al.* (1965) gave a synopsis of Microsporidia of freshwater and euryhaline fishes described till that time. They have also given spore drawings and salient features necessary for identification of the known microsporidia of freshwater and euryhaline fishes. The spores in the present form are monomorphic. A perusal of the literature shows that no microsporidian parasite has been recorded from the present host and as such it is considered new to science for which the name *Pleistophora sauridae* n.sp. is proposed.

*Spore measurements.*—The spores range in size from  $3.6\mu$ – $4.2\mu$  in length and  $2.0\mu$ – $2.2\mu$  in breadth.

*Nosema sauridae* n.sp.

Host: *Saurida tumbil*

Site of infection: Visceral muscles

Type slide: Department of Zoology, Andhra University, Waltair.

As in the case of *Pleistophora*, *Nosema* was found throughout the year and the infection was very light. There does not appear to be any apparent injury to the host.

In sectioned material the cyst wall showed two layers, an outer muscular and an inner connective tissue layer. The connective tissue showed numerous cells with vacuolated cytoplasm. The earlier stages of schizogony are found within the connective tissue cells but the more advanced stages appear to be intercellular (Fig. 2 H). Uninucleate sporonts are found in the lumen of the cyst. They are pyriform in shape with a large nucleus and measure  $2.0\mu \times 1.6\mu$ . The sporonts are usually attached end to end and appear like long chains (Figs. 2 A and E). Each sporoblast gives rise to a single spore, the transformation being completed when a tough transparent wall is formed around each one of them (Fig. 2 D).

The spores are pyriform in shape and the length and breadth of one hundred spores taken at random ranged from  $2.3\mu$ – $3.8\mu \times 1.8\mu$ – $2.0\mu$ . Fresh spores observed under the bright field illumination do not reveal any internal structure other than a single large vacuole which occupies the anterior

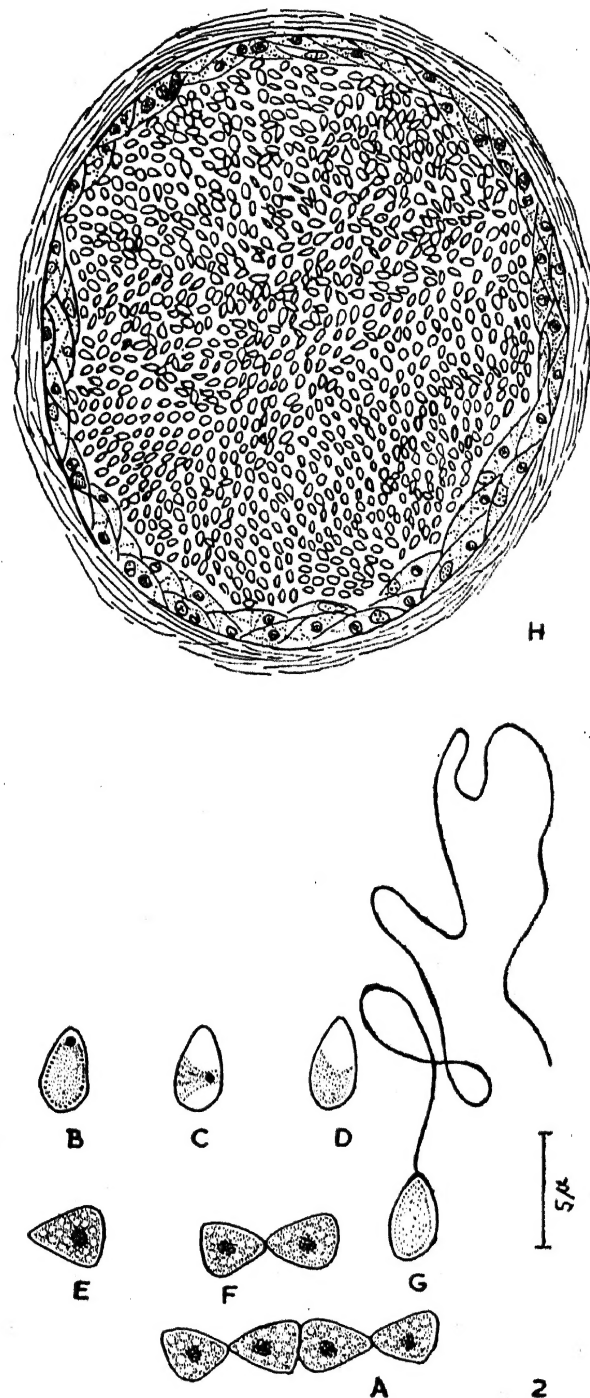


FIG. 2. *Nosema sauridae* n.sp. (A) Sporonts associated in the form of chains. (B) A spore stained according to the PAS technique. Note the dot-like polar cap at the anterior end. (C) A spore stained with Giemsa. Note the sporoplasm extending in the form of a band in the middle of the spore and a dot-like nucleus. (D) A fresh spore. (E) A single sporont. (F) Two sporonts showing end to end attachment. (G) A single spore with extruded polar filament, (H) Section of a cyst showing an outer muscular and an inner epithelial layer.

half of the spore (Fig. 2 D). A posterior vacuole is not clearly seen. The cytoplasm occupies the rest of the area in the spore. Spores stained with Giemsa after an initial hydrolysis in 1 N HCl for ten minutes at 60° C reveal the presence of the nucleus to one side. The polar filament appears in the form of a coiled watch spring (Fig. 2 C). The polar filament is extruded by the addition of a drop of hydrogen peroxide to the wet smear. The polar filament is uniformly thin and when fully extruded measures 150–180  $\mu$  in length. Spores stained according to the PAS technique showed the presence of a small dot-like PAS positive polar cap at the anterior end (Fig. 2 B).

#### DIAGNOSIS

The present parasite is placed in the genus *Nosema* because each sporoblast gives rise to one spore only. A large number of microsporidian parasites belonging to the genus *Nosema* have been reported from a variety of hosts including a few from fishes. This is, however, the first record of the occurrence of species of *Nosema* from the present host for which the name *Nosema sauridae* n.sp. is proposed.

*Spore measurements.*—The spores range in size from 2.3  $\mu$ –3.8  $\mu$  in length and 1.8  $\mu$ –2.0  $\mu$  in breadth.

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